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The Development of Casodex™ (Bicalutamide): Preclinical Studies

Key Words
Androgen antagonists
Antiandrogens
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Casodex™
Drug treatment
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Prostate cancer

Abstract

Casodex™ (bicalutamide, Zeneca Limited) was developed for the treatment of prostate cancer from a series of nonsteroidal compounds related to flutamide. Casodex is a selective antiandrogen that binds to rat, dog and human prostate androgen receptors, and has approximately a 4-fold higher affinity for the rat androgen receptor than hydroxyflutamide, the active metabolite of flutamide. Casodex also binds to androgen receptors found in the LNCaP human prostate tumour and the Shionogi S115 mouse mammary tumour cell line, as well as androgen receptors transfected into CV-1 and HeLa cells. In all cases, Casodex behaves as a 'pure' antiandrogen and inhibits gene expression and cell growth stimulated by androgens. Studies in vivo show that Casodex is a potent antiandrogen in the rat. In contrast to flutamide, which produces dose-related, marked increases in serum luteinising hormone (LH) and testosterone, Casodex has little effect on serum LH and testosterone; that is, it is peripherally selective. The peripheral selectivity of Casodex has now been shown to be due to poor penetration across the blood-brain barrier. In dogs, Casodex has exquisite potency and causes dose-related atrophy of the prostate gland and epididymis; with an oral ED_{50} of 0.1 mg/kg, it is about 50 times as potent as flutamide in this species. Casodex is also peripherally selective in the dog. In addition, magnetic resonance imaging studies have shown that Casodex is a potent antiandrogen in the monkey. Casodex, at a daily oral dose of 25 mg/kg effected a highly significant reduction in the growth of Dunning R3327H transplantable rat prostate tumours that was equivalent to that achieved by either surgical or medical castration with the LH-releasing hormone agonist Zoladex™ (goserelin). In a comparative study, flutamide was shown to be both less potent and less active than Casodex. In these preclinical studies, Casodex was well tolerated. The preclinical properties of Casodex give it advantages, with respect to potency, tolerability and the maintenance of effective antiandrogen serum concentrations, over other available antiandrogens. Moreover, it has a half-life that is compatible with once-daily administration.

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Prostate cancer has become the most commonly diagnosed malignancy and in American men is the second leading cause of cancer-related death [1]. It is, therefore, imperative that new and improved treatment options are made available.

Hunter [2] in 1786 was the first to recognise the importance of testes function to the development of the accessory sex organs including the prostate. Another Scot, Beatson [3] in 1896, observed by analogy that oophorectomy in patients with breast cancer led to tumour regression. It could thus be claimed that the involvement of the testes in prostate tumour growth was obvious as was the likelihood of regression following orchidectomy. However, the definitive proof that prostate cancer was susceptible to androgen withdrawal was only provided in 1941 by the authoritative work of Huggins et al. [4]. Since that time various methods and drugs have been used to induce androgen withdrawal: surgical removal of the testes is the simplest and still most frequently practised technique. Suppression of pituitary gonadotrophin secretion first by oestrogens [5] and later by luteinising hormone-releasing hormone (LHRH) analogues [6, 7] prevents the stimulus required to generate testosterone by the testes. As Bracci and Di Silverio [8] and Labrie et al. [9] have pointed out, such procedures only remove part of the androgenic stimulus to the prostate tumour; that provided by the adrenal gland remains and so only partial androgen deprivation is achieved. Antiandrogens which bind to the androgen receptor and thereby block androgen action, from whatever source, have the potential to effect maximal androgen withdrawal either in combination with surgical or medical castration with LHRH agonists, or if sufficiently potent, as monotherapy.

Currently Available Antiandrogens

Four such antiandrogenic drugs (fig. 1) have now been used clinically in Europe and America and another, the new steroidal compound, WIN 49,596, has been evaluated in extensive preclinical studies [10–12]. All of these drugs have merits and limitations.

Cyproterone acetate, the first antiandrogen to be used clinically, is a tribute to the pioneering work in the early 1960s of Neumann and colleagues at Schering AG in Berlin. Cyproterone acetate is a steroidal antiandrogen which is effective in the majority of patients and produces side effects which are generally less than those seen with oestrogens [13]. However, thrombosis is seen in 5% of patients, fluid retention in 4%, gynaecomastia in 13% and loss of libido in the majority; the steroidal nature of the drug is probably responsible for its cardiovascular side effects and its adverse effect on serum lipoproteins [14].

Because of its steroidal properties, there can also be effects on carbohydrate metabolism so care is advised when prescribing the drug for diabetic patients [15, 16]. In addition, variable degrees of migraine and gastrointestinal upset have been reported [13]. Cyproterone acetate is also a potent progestin. The progestational properties of cyproterone acetate provide an advantage over surgical and medical castration and some 'pure' antiandrogens because the drug does not produce hot flushes. However, cyproterone acetate severely suppresses libido and causes gynaecomastia and loss of erectile potency [17]: it has, indeed, been used to treat sexual deviation. Loss of libido may not be a severe disadvantage in many very old patients but in younger men with prostate cancer who are sexually active, loss of libido would be an unfavourable consequence of therapy. However, when used in combination with medical or surgical castration [8, 9], loss of libido is inevitable so in this situation the progestational and gonadotrophin inhibitory properties of cyproterone acetate have less clinical relevance.

There is continuing controversy over the hepatic toxicity of cyproterone acetate, particularly as to whether it has the propensity to induce liver tumours in man [18–20]. Moreover, cyproterone acetate is not registered for treatment of prostate cancer in the United States.

Flutamide (Eulexin™, Schering-Plough International), the first available nonsteroidal antiandrogen, was discovered by Neri and colleagues. Flutamide is also effective in the treatment of prostate cancer and appears to be better tolerated than cyproterone acetate [13]. Since it is a 'pure' antiandrogen, it does not seem to be associated with the side effects of thromboembolism and fluid retention. Moreover, since it is not a progestin, it does not suppress testis function like cyproterone acetate and it rarely causes loss of libido. Gynaecomastia is the most frequently reported side effect [13], but gastrointestinal intolerance, particularly diarrhoea, can be a troublesome side effect [21, 22]. Reversible liver function abnormalities have been reported in some patients, which can be serious [23]. The active metabolite of flutamide, hydroxyflutamide, also has a short half-life (5.2 h) and so the parent drug needs to be administered three times daily [24], which may lead to problems or compliance with therapy. Since it is a 'pure' antiandrogen there is another

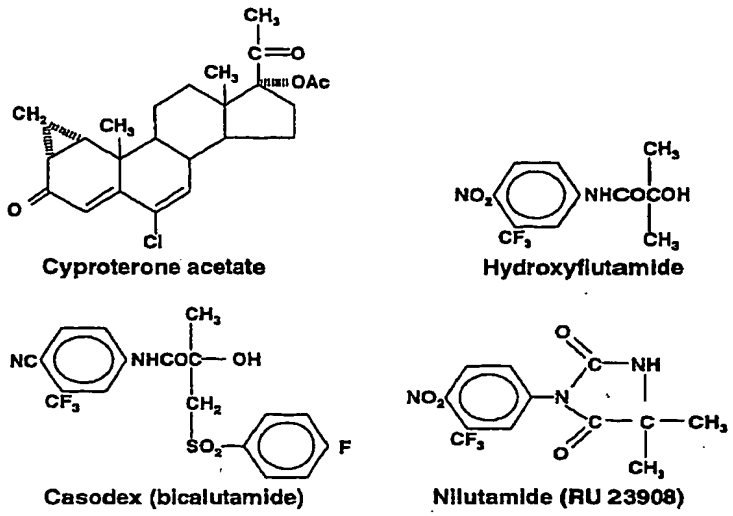

Fig. 1. Structures of antiandrogens.

perceived problem with flutamide: as well as preventing androgen from stimulating prostate growth, it antagonises the action of androgen at the hypothalamus and pituitary gland. This leads to an increase in luteinising hormone (LH) secretion. It is suggested that the resulting increase in output of androgen by the testis requires an increased dose of antiandrogen to neutralise any stimulatory effect on the prostate gland: this is a controversial topic but when given in combination with medical or surgical castration, any rise in serum LH becomes irrelevant to clinical outcome. In addition to elevating androgen secretion, serum oestrogen concentrations are also increased, which would tend to exacerbate any tendency to gynaecomastia.

Nilutamide (Anandron™, Roussel) was the second nonsteroidal 'pure' antiandrogen to become available. It is structurally similar to flutamide, but has a longer half-life (approximately 2 days) [25], so can theoretically be given once daily. Clinical trials with nilutamide have focused primarily on its combination with surgical or medical castration [26, 27]. The side effects of the drug include problems with light/dark adaptation [27–29], alcohol intolerance [27], and interstitial pneumonitis [27], the latter reportedly led to clinical trial cessation in Japan.

The Search for a New and Improved Antiandrogen

Due to the limitations of the currently available antiandrogens, we embarked on a hunt for a new compound. Use as a monotherapy, particularly in younger men, mandated that we sought a 'pure' antiandrogen, since this was likely to maintain libido in a majority of patients. Consequently, our target was a 'pure' antiandrogen that was more potent than flutamide, had a longer half-life and was better tolerated than either flutamide or nilutamide. Specifically, we were challenged by the possibility of finding a peripherally selective antiandrogen that would act at the prostate but would not stimulate the hypothalamic-pituitary axis and thereby induce elevation of circulating LH and testosterone levels (fig. 2). Casodex™ (bicalutamide, Zeneca Limited) was the compound eventually selected from over 2,000 synthesised for this work.

Casodex: Pharmacodynamics

Receptor Binding Studies

Preliminary studies investigated the binding of Casodex to rat prostate cytosol androgen receptors. Displacement curves using non-radiolabelled compounds and the potent selective androgen [^{3}H]-R1881, are shown

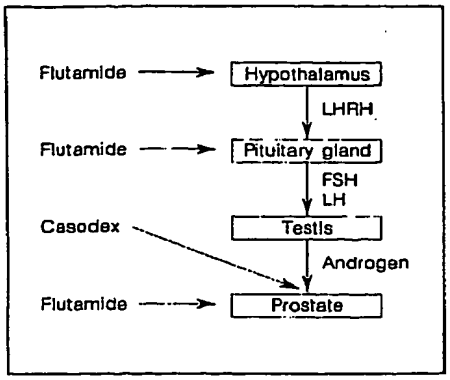

Fig. 2. Effect of flutamide and Casodex at different target organs. Sites of action of antiandrogens. Flutamide acts at the hypothalamus, pituitary gland and prostate. The objective for Casodex was to show peripheral selectivity and block androgen action at the prostate but not at the hypothalamic-pituitary axis.

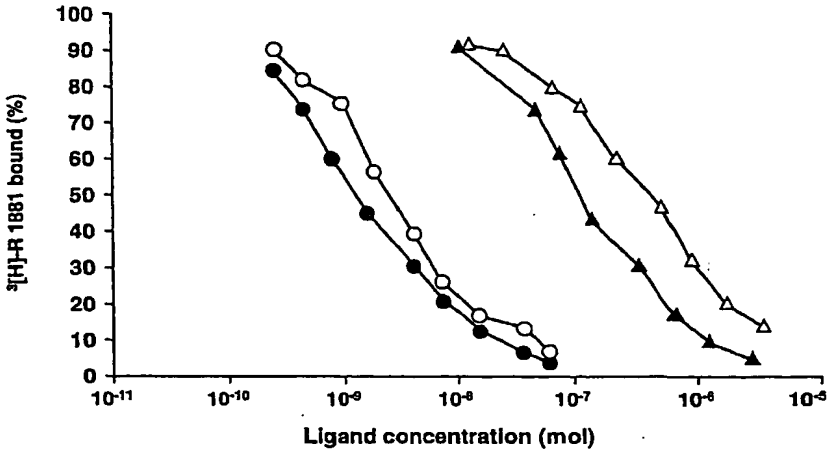

Fig. 3. Displacement of [³H]-R1881 from a rat prostate androgen receptor preparation by increasing the concentrations of unlabelled R1881 (●), 5α-dihydrotestosterone (○), Casodex (▲) and hydroxyflutamide (△).

in figure 3. Both 5α-dihydrotestosterone and R1881 had much higher affinity for the receptor than the two antiandrogens used, with Casodex being superior to hydroxyflutamide. This was reinforced by the IC_{50} values (mass of compound which causes 50% displacement of specifically bound radioligand) for the four compounds and the percentage cross-reactivity of the three synthetic compounds compared with the natural ligand, 5α-dihydrotestosterone (table 1).

These results have been confirmed by Winneker et al. [12], who showed that Casodex had a relative binding affinity of 2% compared with R1881 (100%) after a 1 hour incubation period and 0.43% after 18 h. Similarly, Casodex was shown to bind to a stable transfectant of the rat androgen receptor and mouse mammary tumour virus (MMTV) chloramphenicol acetyltransferase (CAT) and to block the effects of R1881; Casodex alone was devoid of agonist activity [30]. Casodex also binds to rat pituitary cytosol androgen receptors with similar relative affinity (data not shown) as well as to dog and human prostate androgen receptors [11, 31, 32].

Casodex was shown to have negligible affinity for sex hormone-binding globulin and no affinity for corticosteroid-binding globulin. In contrast, cyproterone acetate had significant affinity for sex hormone-binding globulin and high affinity (36%) relative to control (100%) for corticosteroid-binding globulin [31]. Casodex also bound to human androgen receptors transfected into

Table 1. Rat prostate androgen receptor binding data

Compound	IC_{50} nM	% Cross-reaction
R1881	2	190
5α-Dihydrotestosterone	3.8	100
Casodex	190	2
Hydroxyflutamide	700	0.5

COS-1 cells (a fibroblast-like cell line) both in the native form and with a point mutation (codon 868, threonine to alanine) derived from LNCaP human prostate tumour cells [32]. Again Casodex had a low affinity (0.3%) compared with R1881 (100%) and 5α-dihydrotestosterone (33%) but had comparable affinity to hydroxyflutamide (0.4%) and nilutamide (0.1%) for the normal receptor.

It should be emphasised that Casodex has no effect on the activity of steroid 5α-reductase [33].

Antiandrogenic Effects of Casodex in Male Rats

Casodex produced a profound inhibition of androgen-stimulated accessory sex organ growth and at a dose of 10 mg/kg was significantly more active than flutamide in immature castrated rats treated daily with testosterone propionate (0.20 mg/kg). Moreover, dose-response stud-

Table 2. Effect of daily oral doses of Casodex on body, levator ani muscle, seminal vesicles and ventral prostate weights in immature castrated rats treated for 7 days with testosterone propionate (200 µg/kg s.c.)

	Body weight g	Levator ani mg	Seminal vesicles, mg	Ventral prostate, mg
Testosterone	93.0±3.2	29.4±1.2	16.7±0.4	35.5± 2.5
Casodex 2 mg/kg	90.4±2.8	31.2±0.7	7.1±0.3**	21.0±1.5*
Casodex 0.4 mg/kg	93.4±2.5	31.6±2.4	9.7±0.5**	24.6±2.0*
Casodex 0.08 mg/kg	86.8±1.5	27.3±1.0	13.8±1.6	29.7±1.9

*$p < 0.01$; **$p < 0.001$ (Student's t test).

ies showed that Casodex produced a significant inhibition of testosterone propionate-induced accessory sex organ growth at oral doses down to 0.25 mg/kg. These findings are supported by studies in castrated immature male rats given a higher dose of testosterone propionate (0.8 or 1 mg/kg) [10], where Casodex was more potent than flutamide, cyproterone acetate and WIN 49,596, a new steroidal antiandrogen.

In a further study in immature castrated rats given testosterone propionate, Casodex was shown to cause a dose-related reduction in seminal vesicle and ventral prostate weights but surprisingly to have no significant effect on the levator ani (sciatic gastrocnemius) muscle (table 2). Thus, Casodex exerts potent antiandrogenic effects without associated antianabolic effects. If this were to translate to man, then inhibition of prostate tumour growth would be apparent without the antianabolic muscle-wasting response seen following castration.

A comparison of the oral activity of Casodex and flutamide was made in mature male rats (200–220 g) with a 14-day dosing period followed by necropsy on day 15. The doses used and results obtained are shown in figure 4. Casodex reduced the weights of seminal vesicles and ventral prostate glands at all of the dose levels tested but did not cause a significant elevation in either LH or testosterone levels at any dose. In contrast, flutamide caused dose-related increases in both serum LH and testosterone levels. These results have been confirmed and extended in a report by Snyder et al. [10], and in three studies by Chandolia et al. [34, 35] and Simoni et al. [36].

It can be concluded that all of the independent published work confirms the view that Casodex is a potent antiandrogen that is peripherally selective but does not have marked antianabolic activity. In contrast, flutamide has lower potency and no peripheral selectivity in rats.

Explaining the Peripheral Selectivity of Casodex in Rats

A number of possible reasons for the peripheral selectivity of Casodex were apparent. Firstly, there was the possibility that the androgen receptors present in the pituitary gland and hypothalamus are different to prostate receptors. This remains a possibility but molecular biological studies in several laboratories have failed, so far, to demonstrate multiple androgen receptors. Moreover, the data already described demonstrated that Casodex binds as effectively to the pituitary androgen receptor as it does to the ventral prostate receptor. A second explanation could be that when bound to the pituitary androgen receptor some subtle mechanism comes into play which 'masks' the expected antiandrogenic response. Since both Casodex and flutamide are equally effective at sensitising the pituitary gland to the LHRH agonist Zoladex™ (goserelin, Zeneca Limited; fig. 5), this explanation cannot be valid.

A third explanation is that hypothalamic androgen receptors behave differently to peripheral androgen receptors, including those in the pituitary gland. This is unlikely to be the case since Belchetz [37] showed that Casodex increases LHRH pulse frequency in rat hypothalamic fragments in vitro and that both Casodex and hydroxyflutamide increase LHRH pulse frequency following suppression with testosterone. Hence, both of these compounds behave as intrinsic antiandrogens at the hypothalamic androgen receptor.

A further explanation is that Casodex penetrates the blood-brain barrier poorly and thus fails to achieve adequate concentrations in the hypothalamus to exert marked antiandrogenic effects. This is indeed the case as tissue distribution studies with [^{3}H]-Casodex have shown [38] (fig. 6). The tissues studied can be divided into five major groups on the basis of how they sequester radioactivity. Firstly, the organs of metabolism and

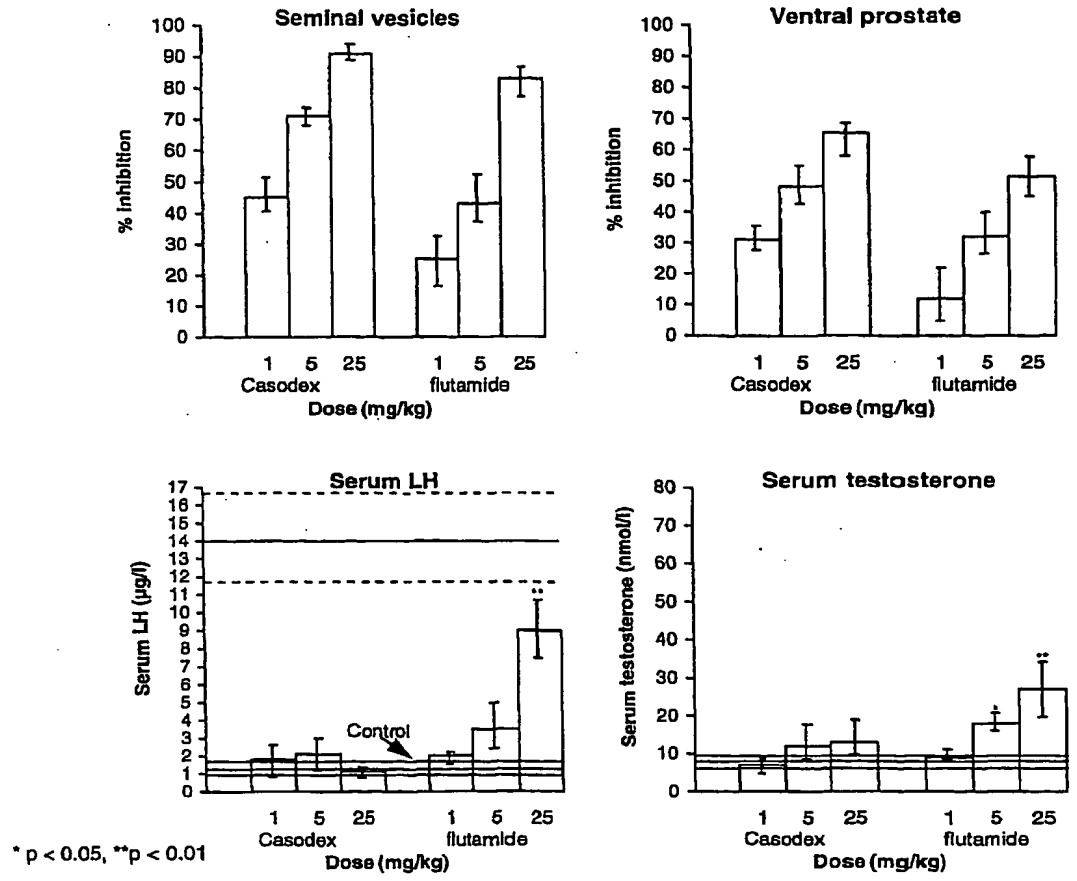

Fig. 4. Comparison of the effects of graded oral doses of flutamide and Casodex administered daily for 14 days on weights of the seminal vesicles and ventral prostate glands and on serum LH and serum testosterone levels in mature male rats. Placebo-treated controls are arbitrarily assigned a value of 0 and surgically castrated controls a value of 100% for measuring inhibition of organ weights. Solid horizontal lines show the range of hormone values for placebo-treated controls and dashed lines the value for surgically castrated controls. *$p<0.05$; **$p<0.01$ compared with placebo-treated control values.

excretion (the liver and kidney); secondly, the androgen target tissues (the prostate gland, seminal vesicles and anterior pituitary gland), thirdly, the non-target organs (spleen and lung); fourthly, the hypothalamus and other central nervous system tissues, and finally the testes. The mean hepatic and renal tissue to serum ratios (TSRs) 1, 5 or 10 h following injection of [^{3}H]-Casodex were significantly higher ($p < 0.05$) than the corresponding means for the prostate gland and spleen. The mean TSRs for the target organs, prostate gland and seminal vesicles were not significantly different from the corresponding splenic mean at any of the three times after injection of [^{3}H]-Casodex. As expected from the in vitro binding studies described earlier there was substantial uptake of [^{3}H]-Casodex by the anterior pituitary gland. The mean testis TSR was seen to be significantly lower ($p<0.05$) than the corresponding splenic mean 1 h after injection. No such significant differences were demonstrable either 5 or 10 h after injection of [^{3}H]-Casodex. The mean hypothalamic and cerebro-cortical TSRs 1, 5 and 10 h after injection were an order of magnitude lower than the corresponding means for any of the other tissues studied,

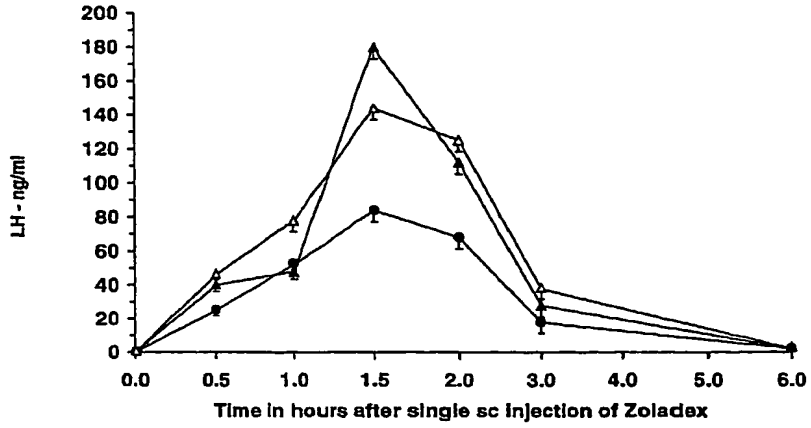

Fig. 5. Effect of 0.1 µg Zoladex s.c. on serum LH in male rats pretreated for 14 days with either 0.5% polysorbate p.o. (●), 5 mg flutamide/kg p.o. (△) or 5 mg Casodex/kg p.o. (▲). The values shown are the means ± SEM (n = 5).

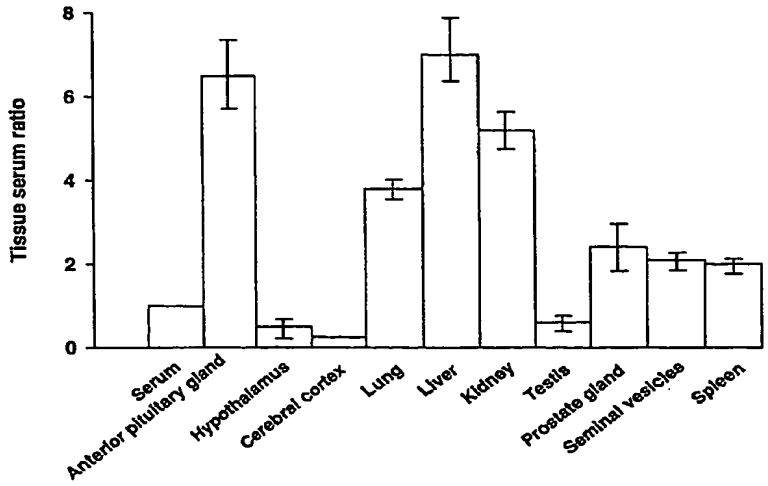

Fig. 6. Distribution of radioactivity in the intact male rat following intravenous injection of [^{3}H]-Casodex. Rats were killed at 1 h and tissues were solubilised and counted for tritium. Tissue radioactivity was expressed as dpm/mg tissue weight and then as a tissue to serum ratio (TSR). Histogram height reflects the mean TSR for each tissue in each group (n = 4 or 5); the vertical lines represent the standard errors of the means.

indicating that Casodex penetrates poorly into the central nervous system. This is almost certainly the explanation for the peripheral selectivity observed in rats.

Endocrine Effects of Casodex in Dogs

The effects of oral administration of Casodex (0.25–4 mg/kg daily for 6 weeks) were evaluated in adult male beagle dogs. No changes in the clinical condition or the behaviour of the animals were noted and the only clinically relevant finding at necropsy was a reduction in size of the testes, epididymides and prostate gland. This observation was confirmed by the assessment of organ weights. Testes, epididymides and prostate weights were all decreased by treatment. There is, however, a large variation in prostate size in control dogs of this age range, so the histological effects give a clearer guide to the efficacy of this drug. Casodex caused atrophy of the prostate gland and epididymides at all dose levels and testis atrophy in 3 of 5 dogs at the highest dose level (4 mg/kg) and in 2 of 5 dogs at 1 mg/kg.

Since 0.25 mg/kg was above the ED_{50} dose for Casodex the study was repeated using an identical protocol except that doses of 0.25, 0.1 and 0.05 mg/kg were used. No clinically relevant findings were noted. At necropsy the weight of the prostate glands in all drug-dosed groups was lower than that of the controls; there were no clear effects on weights of testis or epididymides. Histological examination showed that atrophy of the prostate was dose related with 0.1 mg/kg being an approximate ED_{50}. Atrophy of the epididymides was less marked than that of the prostate and was only seen in tissue from dogs given 0.25 mg Casodex/kg. There were no significant effects on serum testosterone concentrations. Indeed, estimation of serum testosterone concentrations from a toxicity study where daily oral doses of 20 and 100 mg Casodex/kg were given for 28 days (non-blinded) shows that even at these doses, which are very substantial multiples of the ED_{50} value, there were no effects on serum testosterone concentrations.

These results are in accord with the findings of Juniewicz et al. [11] who showed that an oral dose of Casodex (0.25 mg/kg) daily in beagle dogs caused a significant reduction in prostate volume assessed by ultrasound 2 weeks after the start of treatment, and was more potent than the steroidal antiandrogen, WIN 49,596, and the 5α-reductase inhibitor, MK-906. After 16 weeks treatment, Casodex (0.25 mg/kg daily orally) also induced a significant reduction in prostate weights and prostate DNA content compared with intact controls but this dose was not equivalent to surgical castration [11]. Diffuse glandular atrophy was found in dogs treated with Casodex and there was a significant reduction in semen volume after 16 weeks of treatment with Casodex [11]. There was also no effect of Casodex on testes weights at 0.25 mg/kg daily nor on spermatogenesis and Leydig cell appearance. Finally, Casodex was shown to have no effect on serum testosterone concentrations [11].

Effects of Casodex on Accessory Sex Organs in Adult Male Monkeys

It was important to evaluate the effect of Casodex on accessory sex organ size and plasma testosterone concentrations in monkeys, a species more closely related to man. In order to reduce the number of animals used in the study, accessory sex organ sizes were assessed by a sophisticated nuclear magnetic resonance imaging procedure.

In 4 male monkeys *(M. nemestrina)* prostate volume was reduced by about 20% after treatment for 4 weeks with Casodex at 1 mg/kg and by about 30% after treatment with Casodex at 5 mg/kg for a further 2 weeks. Seminal vesicles showed more extensive shrinkage, the corresponding reduction being about 45 and 55%, respectively. Prostate volumes recovered on withdrawal of Casodex. In an untreated animal, the prostate volume fell by 14% and seminal vesicle volume by 16% during the period of daily dosing by gavage.

Plasma testosterone concentrations were measured in the 4 monkeys treated with Casodex and the 1 treated with vehicle and subjected to periodic imaging of the prostate and seminal vesicles. Marked fluctuations were seen in all the animals both during the period of treatment and afterwards but no consistent trends were apparent that could be attributed to Casodex.

Effects of Casodex on Tumour Cells in vitro

An important study by Veldscholte et al. [39] described the effects of Casodex on binding to an androgen receptor present in the human LNCaP prostate tumour cell line. In this tumour line, the androgen receptor has a point mutation in the steroid-binding domain (codon 868, threonine to alanine). This defect does not prevent antiandrogens like cyproterone acetate, nilutamide and hydroxyflutamide from binding to the receptor, but the resulting receptor complex stimulates growth of the cell line: it does not prevent androgen-induced growth. Casodex is unique amongst currently available antiandrogens in that it does not stimulate cell growth. Casodex will, however, inhibit growth in response to the synthetic androgen R1881. In these experiments, hydroxyflutamide only stimulates cell growth (fig. 7).

Detailed molecular biology investigations showed that the heat shock proteins, 90, 70 and 56 co-precipitate with the androgen receptor and so may have a 'chaperon' role. Binding of androgens and the older antiandrogens with the receptor caused dissociation of the heat shock proteins and, in parallel, transformation of the receptor to a tight nuclear-binding form. In contrast, Casodex did not cause dissociation of the heat shock proteins, and transformation of the androgen receptor to a tight nuclear-binding form also did not occur. Whether this observation has any clinical significance must await the outcome of further studies but it is tempting to speculate that Casodex would still be effective in some patients with tumours which fail to respond to primary therapy with other antiandrogens, or that it may be effective in

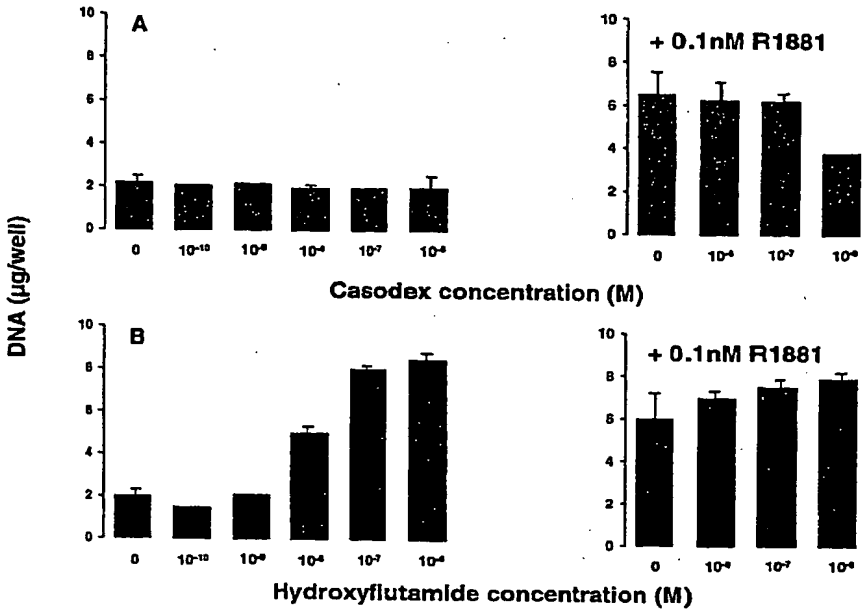

Fig. 7. Effects of Casodex and hydroxyflutamide on growth of LNCaP cells. Various concentrations of Casodex (**A**) and hydroxyflutamide (**B**) were added alone (left panels) or in combination with 0.1 n*M* R1881 (right panels) with one medium change after 3 days. DNA content was determined after 6 days culture. Means and standard deviations of four measurements are shown.

patients with tumours who relapse on such antiandrogen therapy. Evidence that withdrawal responses to flutamide and chlormadinone acetate are not infrequent is now available [40–42]: such tumours might actually be stimulated by these drugs if they arise from cell clones that contain a mutated androgen receptor but would still be responsive to Casodex. Examination of androgen receptors by polymerase chain reaction and sequence analysis from refractory and relapsing patients would help to determine whether this conjecture is valid. However, the position may not be simple since some withdrawal responses have also been observed with Casodex [43, 44], although the magnitude of change in serum prostate-specific antigen in some cases would not be counted as a response by the trial criteria, eg greater than 50% fall or return to the normal range. Perhaps these findings simply emphasise that the mechanism of drug resistance in cancer is neither a single nor simple process.

The Shionogi (SC)-S115 mouse mammary tumour cell line contains androgen receptors and its growth can be stimulated by androgens [45]. Short-term androgen withdrawal results in a change from fibroblastic to epithelial morphology, reduced proliferation rate, increased density regulation and the loss of ability to grow in suspension culture [46]. The long-term growth of these cells in the absence of androgen also results in an ordered, reproducible series of phenotypic changes culminating in loss of both cellular and gene-sensitive markers responsive to androgen. Darbre and King [46] have described the effects of Casodex on this cell line. Casodex (at 10^{-6} *M*) inhibited cell growth stimulation by testosterone (10^{-9} *M*) in monolayer cultures and was particularly effective against testosterone at 10^{-8} *M*. Similarly, Casodex at 10^{-6} *M* prevented the growth of suspension cultures of SC-115 cells in the presence of testosterone (10^{-9} *M*) and the proliferation of mouse mammary tumour virus RNA. Casodex had no agonist activity at 10^{-6} *M* since it failed to promote growth in either monolayer or suspension cultures and the cells were of an epithelial appearance. Moreover, Casodex had no stimulatory effect on MMTV RNA production whereas androgen did cause such a stimulation.

There are also three literature reports of the effects of Casodex on cells transfected with androgen receptors. Fuhrmann et al. [30] stably transfected the rat androgen receptor and a MMTV-CAT gene construct into receptor-negative CV-1 simian cells. In contrast to the potent androgen R1881, neither hydroxyflutamide nor Casodex stimulated CAT activity. Both nonsteroidal antiandro-

gens inhibited, in a dose-dependent manner, CAT expression induced by R1881.

Veldscholte et al. [32, 39] have described the effects of Casodex on a HeLa cell line stably transfected with either the normal human androgen receptor (wild-type) or the receptor with a point mutation (codon 868, threonine to alanine) found in the LNCaP prostate tumour cell line. A glucocorticoid-responsive element linked to CAT, pG29Gtk CAT, was used as a reporter. Casodex failed to stimulate CAT activity in either transfected cell line and inhibited CAT expression induced by R1881. Hydroxyflutamide behaved as a 'pure' antiandrogen in the cell line containing the wild-type construct but stimulated CAT expression in the cell line containing the mutated receptor without being able to inhibit CAT activity induced by R1881. These results are in accord with the findings in the LNCaP cell line [39].

Antitumour Effects of Casodex in Rats

Studies on the antitumour effects of Casodex were carried out in rats bearing the Dunning R3327H prostate tumour. In the first experiment groups of 10 animals were given daily oral doses of either 0.5% polysorbate or Casodex (25 mg/kg). Two further groups of 10 animals were surgically castrated and given identical control and drug treatments. The tumour growth response is shown in figure 8. Tumours in the intact control group grew rapidly. Castration and Casodex treatment caused significant ($p < 0.05$) suppression of growth with all of these treatments producing responses of a similar magnitude; there was no significant difference in tumour growth rate between castrated rats and castrated rats treated with Casodex.

In another experiment, the efficacy of Casodex was compared with flutamide. The drugs were given as daily oral doses at both 25 and 5 mg/kg in groups of 8 animals. In this experiment, dosing was continued until the tumour size in individual animals reached 20 cm^2 when they were killed; serum was collected for radioimmunoassay of follicle-stimulating hormone, LH and testosterone. The treated groups could be compared with the intact control groups for the first 11 weeks of the study only. Casodex caused a significant ($p<0.05$, weeks 4–5; $p<0.01$ weeks 6–11) inhibition of tumour growth rate at both 5 and 25 mg/kg with no difference in the response achieved by either of these doses. Flutamide appeared less effective but produced a significant ($p<0.05$) reduction in tumour size at weeks 6–11 at a dose of 25 mg/kg and at weeks 7–11 at a dose of 5 mg/kg. Even at the higher dose the mean effect was not as great as that seen with Casodex at 5 mg/kg. In this study, an analysis was performed of the time taken for the total tumour size to increase to an ethically acceptable maximum level (≥ 20 cm^2) or to death if this occurred earlier. Survival time was significantly shorter for intact controls than for drug-treated animals: flutamide (25 mg/kg), $p=0.015$; flutamide (5 mg/kg), $p=0.029$; Casodex (25 mg/kg), $p=0.007$; Casodex (5 mg/kg), $p=0.016$. Since the animals were killed at different times during the experiment caution needs to be exercised in interpretation of organ weight data. However, it is clear that Casodex was considerably more potent than flutamide at causing regression of the accessory sex organs. Moreover, Casodex failed to affect serum LH and testosterone, in contrast to flutamide which elevated both hormones even at the lower dose of 5 mg/kg.

In view of the continuing interest in the concept of 'total androgen withdrawal' [8, 9], a comparison was made of the effects of treatment with single subcutaneous biodegradable depots containing 1 mg of the LHRH agonist Zoladex [47] given every 28 days, daily oral administration of Casodex (25 mg/kg) and a combination of the two therapies in rats bearing Dunning prostate tumours. The results are shown in figure 9 and clearly indicate that both Zoladex and Casodex treatment alone are as effective as surgical castration at inhibiting the growth rate of these prostate tumours. Furthermore, there was no advantage of the combination therapy over the single therapies. Zoladex produced the expected castration effect on serum testosterone and accessory sex organ weights which was not altered by the addition of Casodex. Since adrenal androgen contributes little to circulating androgens in the male rat, these findings should not be used to cast doubt on the validity of the total androgen withdrawal hypothesis. The endocrine data were entirely consistent with the previous findings. Casodex caused a highly significant reduction in accessory sex organ weights but these did not quite achieve castrate values. Serum follicle-stimulating hormone was slightly stimulated by treatment with Casodex but there was no significant effect on serum LH, prolactin and testosterone.

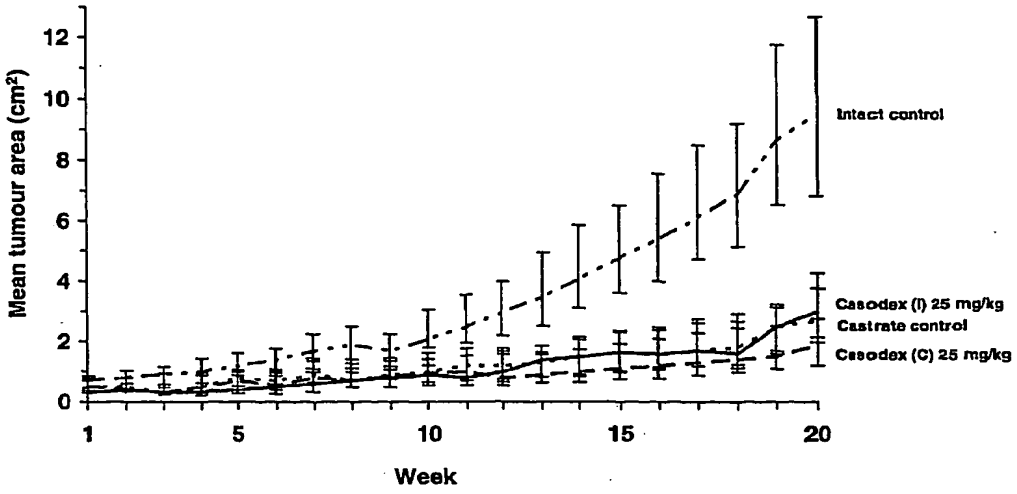

Fig. 8. Effect of a daily oral dose of 25 mg Casodex/kg on the growth of Dunning prostate tumours: I = intact; C = castrate.

Pharmacology and Toxicology of Casodex

In all studies, Casodex was well tolerated. Casodex had no significant effects in any general pharmacology tests except in the dog where, on chronic administration, it caused a small increase in heart rate and a reduction in P-R interval at doses more than 25 times the ED_{50} value for prostate atrophy. Since there was neither impairment of cardiac function nor pathological findings when cardiac histology was examined in chronic toxicity studies (see below), it was concluded that this idiosyncratic finding is of no toxicological consequence. This conclusion has been supported by clinical observations which failed to demonstrate any effect of Casodex on cardiac function.

Casodex was shown to be a selective antiandrogen and specifically possessed neither oestrogenic nor antioestrogenic, progestational nor antiprogestational, neither glucocorticoid nor antiglucocorticoid and neither mineralocorticoid nor antimineralocorticoid activity in classical endocrine tests. Casodex also showed no androgenic activity.

Casodex has been studied in both acute and chronic toxicity studies. It is well tolerated and, apart from effects related directly to its mode of action as an antiandrogen, only two findings require comment.

In toxicity studies in dogs, Casodex caused a dose-related increase in heart rate and decrease in P-R interval

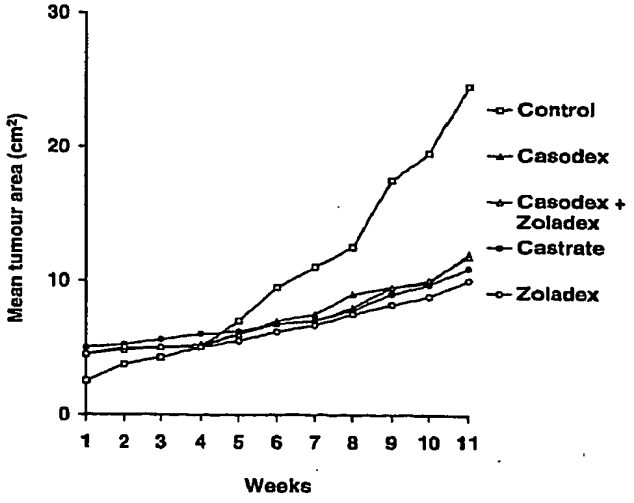

Fig. 9. Effect of single s.c. 1 mg Zoladex depots given every 28 days, daily oral administration of 25 mg Casodex/kg and the combination of both treatments on the growth of Dunning prostate tumours. The effects of surgical castration at the start of the experiment are also shown.

consistent with the findings in the pharmacology tests. There were no pathological findings related to this physiological change and there was no impairment of cardiac function. In clinical studies [48], there were no effects on cardiac function so the change is considered to be of no relevance to man.

In toxicity studies in rats, benign adenomas of the Leydig cells and thyroid were found as well as some liver tumours [49]. Leydig cell hyperplasia and adenomas are common features following alteration of the endocrine milieu in rats but are of no relevance to human exposure to the drug. This is consistent with a lack of Leydig cell hyperplasia or adenomas in testes from patients treated with Casodex [50]. The liver and thyroid tumours are related and considered to be due to the mixed function oxidase-inducing properties of Casodex in the rat. It is well recognised that agents which induce mixed function oxidases in the rat can lead to liver and thyroid tumours but that this is of no relevance to man [51, 52].

Pharmacokinetics

A major objective of the research programme that led to the development of Casodex was pharmacokinetic properties compatible with once-daily dosing in order to improve the likelihood of compliance with therapy. Cockshott et al. [53] have shown that Casodex has an elimination half-life of 1 day in the rat and 6 days in the dog so amply fulfils the criterion set for selection. This means that once-daily administration will give stable blood levels that will antagonise circulating androgen throughout the day.

Conclusions

Casodex is a potent, orally active, well-tolerated antiandrogen with a half-life that is compatible with once-daily administration. In rats it is not antianabolic at doses which cause potent suppression of prostate growth. The preclinical properties of Casodex give it advantages over other available antiandrogens in terms of potency, tolerability and the maintenance of effective antiandrogen serum concentrations. Casodex thus provides a clear alternative to other antiandrogens as both monotherapy and in combination with medical and surgical castration for the treatment of prostate cancer.

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